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L-Type Calcium Channels Mediate Dynorphin Neuropeptide Release from Dendrites but Not Axons of Hippocampal Granule Cells

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Summary

Granule cells in the guinea pig dentate gyrus release κ opioid neuropeptides, dynorphins, from dendrites as well as from axon terminals. We have found that both L- and N-type calcium channel antagonists inhibited dendritic dynorphin release. In contrast, N-type but not L-type calcium channel antagonists inhibited axonal dynorphin release. Neither L- nor N-type channel antagonists directly altered the effects of κ opioid receptor activation. By inhibiting dynorphin release, L-type channel antagonists also facilitated the induction of long-term potentiation of the perforant path–granule cell synapse. These studies establish that a single cell type can release a transmitter from two different cellular domains and provide new distinction between axonal and dendritic transmitter release mechanisms.

Introduction

Much attention has been focused on the important role of specific voltage-sensitive calcium channels in mediating neurotransmitter release and action. For example, excitatory amino acid (e.g., glutamate) release in the hippocampus is mediated by dihydropyridine-insensitive calcium channels, including N-, P-, and Q-type channels (Luebke et al., 1993; Takahashi and Momiyama, 1993; Castillo et al., 1994; Wheeler et al., 1994). The L-type, dihydropyridine-sensitive channels are also expressed in the hippocampus (Ahlijanian et al., 1990; Westenbroek et al., 1990; Hell et al., 1993), yet a role for these channels in endogenous neurotransmitter release has not been demonstrated. In addition, little is known about the types of calcium channels mediating endogenous neuropeptide release in the central nervous system.

Hippocampal dynorphin neuropeptides are contained primarily in dentate gyrus granule cells and have a high affinity for the κ subtype of opioid receptor (Chavkin et al., 1982; Corbett et al., 1982). Endogenous dynorphins can be released by high frequency stimulation (Wagner et al., 1991), and they act at presynaptic κ receptors to inhibit excitatory neurotransmission (Wagner et al., 1992; Simmons et al., 1994) and induction of long-term potentiation (LTP; Wagner et al., 1993; Weisskopf et al., 1993; Terman et al., 1994). The source of dynorphin in the molecular layer has been shown to be the granule cell dendrites, which contain the majority of dynorphin-immunoreactive dense-core vesicles (Drake et al., 1994). Dynorphins released from granule cell dendrites act as retrograde inhibitors of glutamate release from perforant path terminals. In the CA3 region, dynorphins are released from and act upon granule cell axon (i.e., mossy fiber) terminals (Weisskopf et al., 1993), again depressing excitatory transmission by presynaptic inhibition of glutamate release. Here we used the spatial separation of the granule cell dendrites and axon terminals (see Figure 1A) to investigate the roles of L- and N-type calcium channels in controlling dynorphin release at each site.

Results

Dynorphin Release from Granule Cell Dendrites

In the guinea pig hippocampal slice, stimulation of perforant path afferents evoked a field excitatory postsynaptic potential (fEPSP) that was recorded in the dentate gyrus molecular layer. High frequency stimulation (HFS) of the granule cell processes in the hilus produced a transient depression of the fEPSP. A second HFS (HFS 2) in the same slice produced a depression of similar magnitude and duration (Figure 1C). Application of the selective κ receptor antagonist norbinaltorphimine (nBNI; 100 nM) significantly attenuated the depression (Figure 1D). This result was consistent with our previous reports (Wagner et al., 1993; Drake et al., 1994; Terman et al., 1994), and it indicated that the HFS-induced depression was mediated by endogenous dynorphins.

When HFS 2 was delivered in the presence of the L-type calcium channel antagonist nifedipine (10 μM), depression of the fEPSP was also significantly reduced (Figure 1E). Similar results were obtained with 5 μM isradipine, another dihydropyridine L-type channel antagonist ($n = 5$; data not shown), and with a lower dose of nifedipine (1 μM; $n = 2$). Neither nBNI nor nifedipine affected the fEPSP amplitude itself, indicating no direct effect on glutamatergic transmission. Furthermore, combined treatment with nBNI and nifedipine had no greater effect than either drug alone ($n = 6$; data not shown), suggesting that the action of nifedipine was not independent of the action of nBNI. These results indicate that L-type channels mediated the HFS-induced depression of the fEPSP.

Application of the N-type calcium channel toxin ω-conotoxin GVIA (CgTx; 1 μM; Plummer et al., 1989) caused a significant reduction in the perforant path–evoked fEPSP amplitude, to 78.7% ± 2% (mean ± SEM; $n = 18$; $p < .05$) of the control amplitude. CgTx also significantly reduced the HFS-induced depression (Figure 1F). Furthermore, combined treatment with CgTx and nBNI did not have a greater effect than either drug alone ($n = 4$; data not shown), suggesting that CgTx, like nifedipine, was acting on the same inhibitory process as nBNI. These results indicate that N-type channels were also required for the HFS-induced inhibition of the fEPSP.

Because a small component of the HFS-induced depression (typically <1 min) was not sensitive to nBNI or the calcium channel blockers, a subtraction analysis was

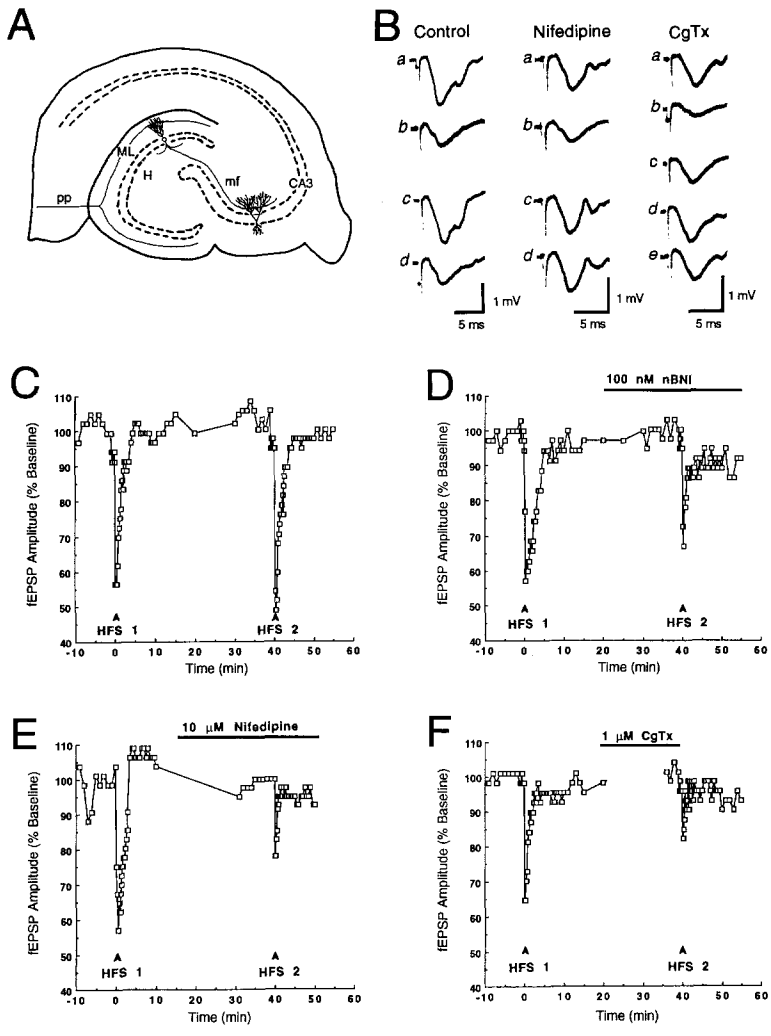

Figure 1. Nifedipine Inhibited the Hilar HFS-Induced Depression of the fEPSP in the Dentate Molecular Layer

(A) Diagram of a hippocampal slice, showing a dentate granule cell (circle) with dendrites in the molecular layer (ML), mossy fiber (mf) axons projecting to stratum lucidum of CA3, and axon collaterals in the hilus (H) and a CA3 pyramidal cell (triangle) with apical and basal dendrites. Perforant path (pp) afferents to the dentate gyrus are indicated. Dotted lines outline stratum granulosum in the dentate gyrus and stratum pyramidale in CA1 and CA3.

(B) Representative traces. Control and Nifedipine: (a) before HFS 1; (b) 1 min after HFS 1; (c) before HFS 2; (d) 1 min after HFS 2. CgTx: (a) before HFS 1; (b) 1 min after HFS 1; (c) after CgTx treatment; (d) before HFS 2, with stimulation intensity increased to restore the fEPSP to the pre-CgTx amplitude; (e) 1 min after HFS 2.

(C–F) Representative time courses. Time points are plotted at 1 min intervals before HFS, at 10 s intervals for 0–3 min, at 30 s intervals for 3–10 min, and at 1 min intervals for 11–15 min after HFS. Data are expressed as a percentage of the mean of five pre-HFS fEPSP amplitudes (% Baseline). The duration of drug application is indicated by a bar in each panel.

(C) Representative time course from a control experiment. In the absence of drug treatment, the second HFS-induced depression was similar in magnitude and duration to the first.

(D) In the presence of 100 nM nBNI, the HFS-induced depression was greatly attenuated. The nBNI-insensitive depression typically lasted <1 min and was similar to the nBNI-insensitive depression reported previously (Drake et al., 1994).

(E) In the presence of 10 μM nifedipine, the HFS-induced depression was greatly attenuated. Nifedipine had no effect on pre-HFS fEPSP amplitudes.

(F) After treatment with 1 μM CgTx, the HFS-induced depression was greatly attenuated. CgTx decreased the perforant path–evoked fEPSP amplitude by an average of 22%, and the stimulation intensity was increased before HFS 2 to restore the fEPSP to its pre-CgTx amplitude. In 5 of 8 slices, HFS in the presence of CgTx produced a large, non-opioid-mediated increase in the fEPSP amplitude (data not shown). This facilitation was prevented by APV. Thus, in the experiment shown, 50 μM APV was included in the perfusion buffer. APV did not alter the HFS-induced depression or the effect of nBNI.

performed to determine the components of the HFS-induced depression that were blocked by each drug. For each experiment, the drug-insensitive depression (after HFS 2) was subtracted from the total depression (after HFS 1), revealing the drug-sensitive component of the depression (Figure 2). In control experiments, the mean depression after HFS 2 was similar to that after HFS 1, and thus the difference was near zero. In contrast, in the drug treatment groups, the mean depression after HFS 2 was significantly smaller than the (predrug) depression after HFS 1. By this analysis, results from nBNI experiments showed that κ receptor activation inhibited the fEPSP amplitude by 20.1% ± 5%, 14.1 ± 3%, and 10.6 ± 2% at 1, 2, and 3 min after HFS, respectively. The depression mediated by L-type channels, i.e., blocked by nifedipine, was 28.6% ± 4%, 22.9 ± 6%, and 14.5 ± 6% of the fEPSP amplitude at 1, 2, and 3 min after HFS, respectively. Similar results were obtained when isradipine was used to block L-type channels (28.7% ± 5%, 26.5% ± 4%, and 22.8% ± 8% at 1, 2, and 3 min after HFS, respectively). N-type channel–dependent events inhibited the fEPSP amplitude by 29.6% ± 8%, 17.0% ± 6%, and 15.2% ± 7% at 1, 2, and 3 min after HFS, respectively. In short, the nBNI-, dihydropyridine-, and CgTx-sensitive components of the depression were similar in magnitude and duration. The drug treatment groups were significantly different ($p < .05$) from the control group, but not from each other. These effects of the dihydropyridines and CgTx demonstrate that L-type channels and N-type channels were required for either dynorphin release or κ receptor action.

To distinguish whether the effects of nifedipine and

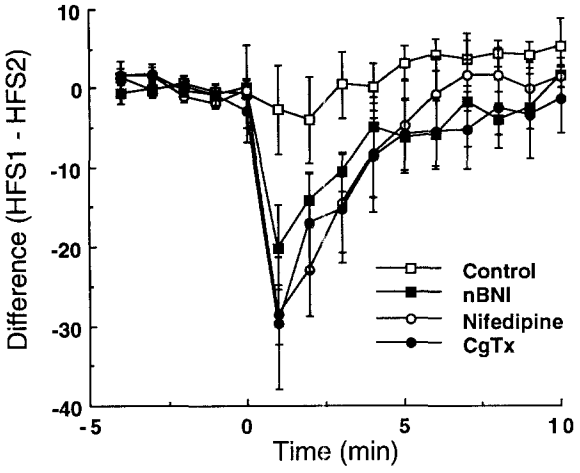

Figure 2. The Dynorphin-Mediated Depression Was Blocked by κ Receptor and Calcium Channel Antagonists

Whereas controls showed nearly identical depressions following HFS 1 and HFS 2, the nBNI, nifedipine, and CgTx groups showed time-dependent, drug-induced differences in post-HFS depressions (HFS 1 minus HFS 2). The component of the response mediated by dynorphin, L-type channels, or N-type channels was defined by the difference in the presence of 100 nM nBNI, 10 μM nifedipine, or 1 μM CgTx, respectively. Time points are plotted at 1 min intervals before and after HFS. Data are mean differences ± SEM (control, n = 11; nBNI, n = 10; nifedipine, n = 6; CgTx, n = 6). The control group included four experiments with APV in the perfusion buffer; the nBNI group also included four experiments with APV; all six experiments in the CgTx group included APV. All three drug-treatment groups were significantly different from the control group ($p < .05$), but did not differ from each other.

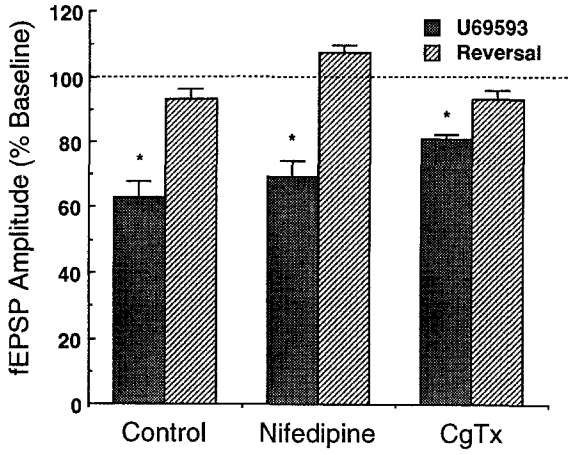

Figure 3. The Calcium Channel Antagonists Did Not Alter κ Receptor Activation

Application of the κ receptor agonist U69593 (1 μM) significantly reduced the fEPSP amplitude. The inhibitory effect of U69593 was reversed by drug washout or by 100 nM nBNI. Treatment of slices with 10 μM nifedipine or 1 μM CgTx did not significantly alter the inhibition produced by U69593, and the effect of U69593 was reversed by 100 nM nBNI. Values are means ± SEM (control, n = 8; nifedipine, n = 7; CgTx, n = 7). Asterisks mark significant ($p < .05$) difference from reversal.

CgTx were caused by an inhibition of dynorphin release or of dynorphin activation of κ receptors, the effect of the calcium channel blockers on κ receptor activation by the selective agonist U69593 was tested (Figure 3). Treatment of hippocampal slices with 1 μM U69593 significantly decreased the fEPSP amplitude to 63.0% ± 5% of control (n = 8). This inhibition was reversed by subsequent application of 100 nM nBNI. The inhibitory effect of U69593 was not altered in slices pretreated with 10 μM nifedipine (69.0% ± 5%; n = 7) or CgTx (80.2% ± 2%; n = 7). The failure of nifedipine and CgTx to block the effects of κ receptor activation suggests that these channel blockers inhibited the HFS-induced depression by inhibiting endogenous dynorphin release in the molecular layer. Therefore, we conclude that both L- and N-type calcium channels were required for the HFS-induced release of dynorphins from granule cell dendrites.

Dynorphin Release from Mossy Fiber Terminals

We also investigated endogenous dynorphin release in the CA3 region. Prior to producing the dynorphin-mediated depression, LTP was induced in the presence of the N-methyl-D-aspartate receptor antagonist ±2-amino-5-phosphonovaleric acid (APV). As circuitry in this region is complex, the insensitivity of LTP to APV confirmed that the responses were evoked by selective mossy fiber stimulation (Claiborne et al., 1993). Also, by inducing a maximal LTP at the onset of the experiment, the inhibitory effects of dynorphin release evoked by subsequent HFS were not obscured by further potentiation. The mean fEPSP potentiation was 70% ± 7% (n = 23) and was not significantly different between drug treatment groups (data not shown).

At 30 min after LTP induction, HFS of granule cell axons produced a brief posttetanic potentiation, followed by a reproducible decrease in the fEPSP amplitude, as shown in Figure 4A. The depression was blocked by 100 nM nBNI (Figure 4B), indicating its mediation by dynorphins. Unlike the results in the dentate gyrus, the depression was not significantly affected by 10 μM nifedipine (Figure 4C). Neither nBNI nor nifedipine altered the fEPSP amplitude. In contrast, CgTx both reduced the fEPSP amplitude by 51% ± 2% (n = 6; data not shown) and blocked the HFS-induced depression (Figure 4D). As in the dentate gyrus, the effect of CgTx and nBNI combined was not greater than the effect of either drug alone (n = 4; data not shown).

U69593 was applied to CgTx-treated slices to determine whether CgTx was occluding dynorphin action by inhibiting κ receptor–dependent events (Figure 5). U69593 reduced the fEPSP amplitude to a similar extent in control and CgTx-treated slices (73.0% ± 4% [n = 5] and 78.6% ± 3% [n = 7], respectively), demonstrating that κ receptor–mediated inhibition was not dependent on CgTx-sensitive calcium channels. These results indicate that CgTx blocked dynorphin release, not its action. Thus, dynorphin release from granule cell axons required N-type, but not L-type, calcium channels, whereas release from dendrites required both L- and N-type channels.

LTP in the Dentate Gyrus Molecular Layer

Previously, we have shown that induction of LTP in the dentate gyrus molecular layer can be inhibited by dynor-

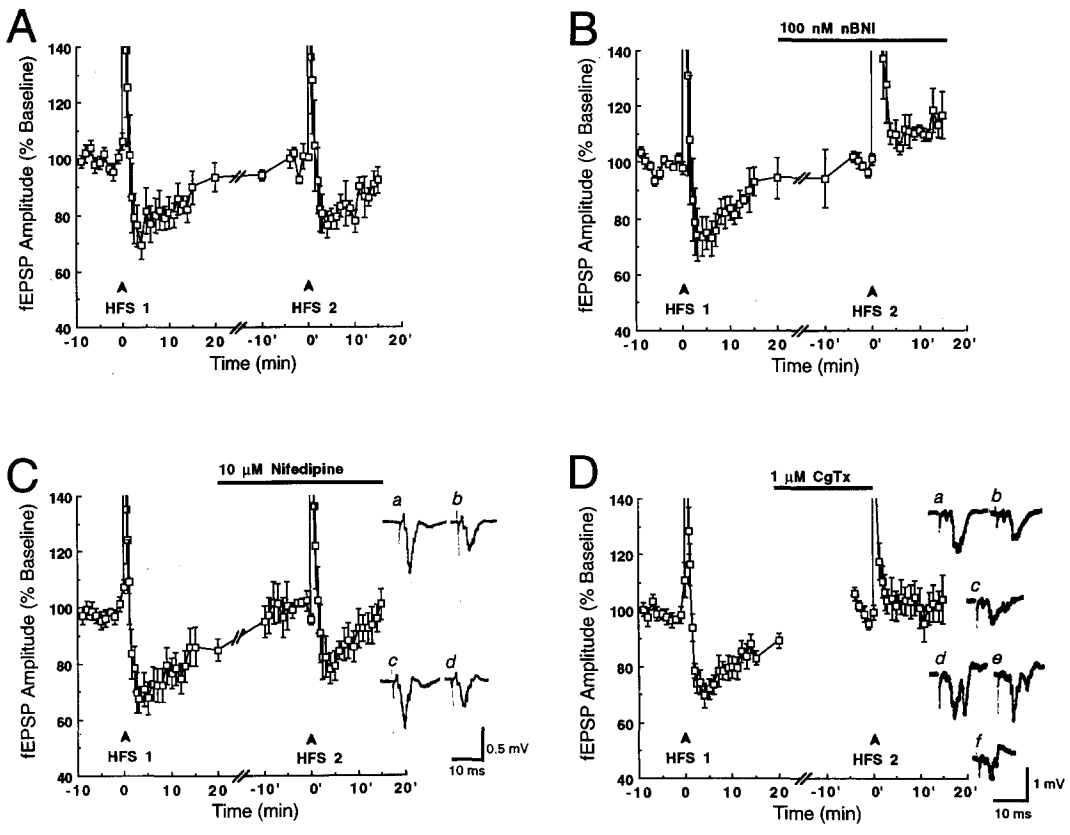

Figure 4. CgTx but Not Nifedipine Inhibited Dynorphin Release in CA3 Evoked by Mossy Fiber HFS

HFS 1 produced posttetanic potentiation (shown truncated at 140%), followed by a depression that was reproduced by HFS 2 in the absence of drug (A; n = 6). The inhibitory effect of HFS 2 was significantly ($p < .05$) attenuated by treatment with 100 nM nBNI (B; n = 5) but was not significantly affected by 10 μM nifedipine (C; n = 6). Insets in (C) show representative traces from a nifedipine experiment: (a) before HFS 1; (b) 4.5 min after HFS 1; (c) before HFS 2; (d) 4.5 min after HFS 2. The HFS-induced depression was significantly ($p < .05$) attenuated by treatment with 1 μM CgTx (D; n = 6). Insets in (D) show representative traces from a CgTx experiment: (a) before HFS 1; (b) 3 min after HFS 1; (c) with 1 μM CgTx ; (d) after increasing stimulation intensity, before HFS 2; (e) 3 min after HFS 2; (f) with 10 μM CNQX. After CgTx treatment, variable after-discharges occasionally developed (d and e). The initial amplitude, however, was consistent, and therefore it was the component measured in these experiments. Values represent means ± SEM.

phins released from granule cells (Wagner et al., 1993; Terman et al., 1994). We compared the effects of nifedipine on LTP induced by two different perforant path HFS paradigms, one that simultaneously releases dynorphins (500 ms duration) and one that does not (20 ms duration). The inhibitory role of dynorphins was inferred by the potentiating effects of nBNI in the former but not the latter LTP paradigm (Terman et al., 1994) (Figure 6). Like nBNI, nifedipine significantly enhanced LTP induced by the 500 ms paradigm, but it had no significant effect on LTP induced in the absence of dynorphin release (20 ms). Again, the effects of nifedipine and nBNI were not additive. The facilitation of LTP by nifedipine was not due to a direct effect on LTP induction mechanisms, because the LTP induced by the 20 ms paradigm, which does not cause dynorphin release, was not affected. Furthermore, the facilitation by nifedipine was not due to dynorphin-independent mechanisms, because the effects of nifedipine and nBNI were not additive. Thus, the effect of nifedipine on LTP was likely due to inhibition of L-type channel–mediated dynorphin release. By mediating dynorphin release from granule cell dendrites, L-type cal-

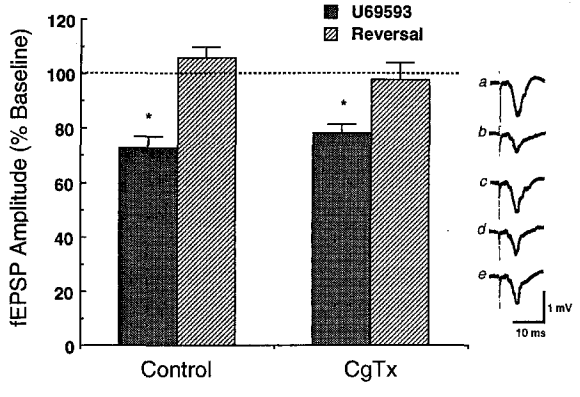

Figure 5. In CA3 the Inhibitory Effect of 1 μM U69593 Was Not Significantly Affected by 1 μM CgTx but Was Reversed by 1 μM nBNI

Values are means ± SEM for control (n = 5) or CgTx (n = 7). Asterisks mark significant ($p < .05$) difference from reversal. Representative traces: (a) before CgTx; (b) after CgTx; (c) stimulation intensity increased to restore the fEPSP to its pre-CgTx amplitude; (d) after U69593; (e) after nBNI.

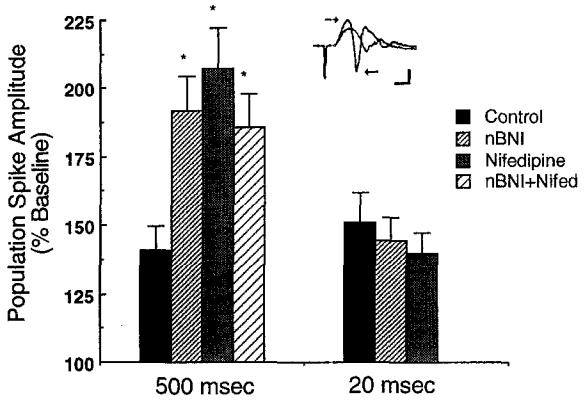

Figure 6. Facilitating Effect of Nifedipine on Dynorphin-Modulated but Not Dynorphin-Independent Paradigms of LTP Induction in the Dentate Gyrus

Following the 500 ms stimulation paradigm, LTP was reliably induced in no-drug control slices. LTP was significantly ($p < .05$) increased in slices perfused with nBNI (100 nM), consistent with our previous findings (Terman et al., 1994). Nifedipine (10 μM) also significantly increased LTP ($p < .05$). The combination of nBNI and nifedipine had no greater potentiating effect than each drug given individually. Following the 20 ms stimulation paradigm, a potentiation of similar magnitude to that induced by the 500 ms LTP paradigm was seen in no-drug controls. In contrast to the 500 ms paradigm, however, neither nBNI nor nifedipine had any effect on LTP induced by the 20 ms paradigm. The inset shows representative traces of population spike responses in the presence of nifedipine (amplitude indicated by arrows), before and 30 min after LTP induction. Calibration bars indicate 0.5 mV (vertical) and 2.5 ms (horizontal). Data shown are percentage baseline values and represent mean ± SEM ($n = 6$ in each group). Asterisks mark significant ($p < .05$) difference from no-drug controls.

cium channels may play an important role in regulating synaptic plasticity in the hippocampus.

Discussion

In these studies, we used HFS-induced depression of fEPSP amplitudes as a sensitive and reliable, though indirect, measure of endogenous dynorphin release. The validity of this measure is based on observations that the depression is sensitive to both nBNI (Wagner et al., 1993; Weisskopf et al., 1993; Drake et al., 1994) and anti-dynorphin antibodies (Wagner et al., 1991, 1993), is mimicked by exogenous dynorphins (Wagner et al., 1992; Weisskopf et al., 1993), and is produced by stimuli that release dynorphins in neurochemical assays (Wagner et al., 1991). Because our measure of dynorphin release is based on inhibition of glutamate release, manipulations that largely block the fEPSP, such as ω-agatoxin IVA inhibition of P-type calcium channels (Castillo et al., 1994), could not be used. On the other hand, antagonizing L- and N-type channels had little or no effect on glutamate release, but effectively blocked dynorphin release.

L-Type Calcium Channels

In the dentate gyrus molecular layer, HFS caused the release of endogenous dynorphins from granule cell dendrites, resulting in inhibition of both the fEPSP amplitude and LTP induction. This HFS-induced depression was inhibited by nifedipine. Further experiments revealed the basis of this action of nifedipine. The effects of nifedipine were mediated by the dynorphin system, because combined treatment with nBNI and nifedipine did not have a greater effect than either drug alone. Nifedipine did not inhibit κ receptor–dependent events, because the response to U69593 was unchanged by nifedipine. Furthermore, the blocking effect of nifedipine was not an artifact of the antidromic HFS design, because nifedipine was also effective in experiments using orthodromic HFS (see Figure 6). Thus, we conclude that nifedipine blocked dynorphin release from granule cell dendrites. These physiological findings parallel the anatomical localization of class C, L-type channels on granule cell dendrites (Hell et al., 1993), and our results show that these channels are involved in neurotransmission. L-type channels have been reported to mediate the release of several endocrine peptides (Blotner et al., 1990; Miller, 1990; Scherubl et al., 1993). The present study demonstrates a role for these channels in mediating peptide release in the central nervous system as well.

By decreasing dynorphin release, nifedipine also facilitated LTP induction. LTP in the hippocampus is thought to be a cellular model of learning (Bliss and Collingridge, 1993). Our observation that blocking L-type channels in the dentate gyrus inhibited dynorphin release and thereby enhanced LTP provides a possible explanation for the reports that dynorphins (Jiang et al., 1989; Zhang et al., 1991), L-type channel activation (Levy et al., 1991), and granule cell HFS (Collier et al., 1987) each impair spatial learning.

N-Type Calcium Channels

Unlike nifedipine, CgTx reduced glutamate release from both perforant path terminals in the dentate gyrus and mossy fiber terminals in the CA3 region. The effect on glutamate release from perforant path terminals was considerably smaller than the effect on glutamate release from mossy fibers (21% versus 51%), suggesting that other (non-N-type, non-L-type) calcium channel subtypes play a greater role in glutamate release from perforant path terminals as compared with mossy fibers. Indeed, the P-type calcium channel toxin ω-agatoxin IVA greatly reduced perforant path–evoked responses (unpublished data). Thus, it is likely that glutamate release in the dentate gyrus is dependent on multiple types of calcium channels, as has been shown for glutamate release in CA3 (Castillo et al., 1994) and CA1 (Luebke et al., 1993; Wheeler et al., 1994).

CgTx blocked dynorphin release from both dendrites and axons. Our finding that dynorphin release from mossy fibers was dependent on N-type but not L-type channels is consistent with previous findings in synaptosomal release experiments (Terrian et al., 1989) and in studies of the roles of these channels in LTP. For example, in CA3, CgTx but not nifedipine enhances the early phase of LTP (Castillo et al., 1994). As LTP in CA3 can be inhibited by dynorphins (Weisskopf et al., 1993), CgTx may enhance LTP by inhibiting dynorphin release in CA3, a process that is

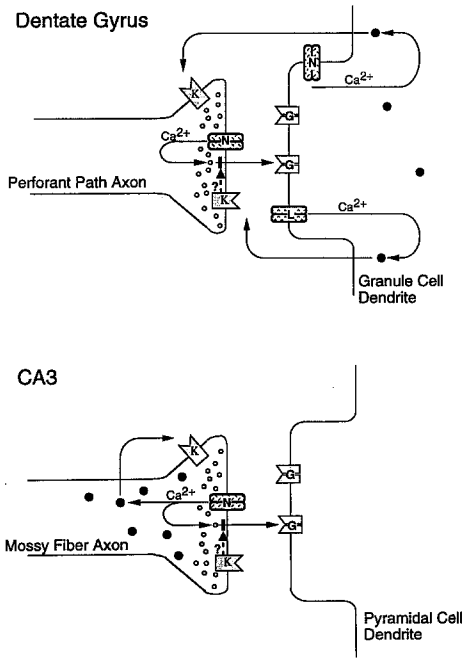

Figure 7. Schematic Illustration of the κ Opioid System in the Dentate Gyrus and CA3 Area of the Hippocampus

Excitatory transmission is mediated by excitatory amino acids such as glutamate, which are contained in small clear vesicles (small, open circles). Glutamate is released in response to calcium influx through multiple types of voltage-sensitive calcium channels, including N-type channels. Released glutamate interacts with postsynaptic glutamate receptors (G). In the dentate gyrus, dynorphin-containing dense-core vesicles (large, closed circles) are released from granule cell dendrites. Calcium influx through dendritic L- and N-type channels is required for dynorphin release. The released dynorphins act at κ opioid receptors (K) on perforant path terminals and inhibit glutamate release by an unknown mechanism. In the CA3 region, both glutamate and dynorphin are released from mossy fiber terminals. N-type but not L-type calcium channels mediate both dynorphin and glutamate release. Dynorphins act at κ opioid receptors on mossy fiber terminals to inhibit glutamate and possibly dynorphin release.

analogous to the LTP facilitation by nifedipine in the dentate gyrus reported here. Our results are further supported by anatomical studies, which have reported that N-type channels are located on mossy fibers in CA3 and on dendrites in the dentate molecular layer (Westenbroek et al., 1992). The ability of CgTx to inhibit glutamate release, as well as dynorphin release, in the molecular layer suggests that N-type channels in this area are located both pre- and postsynaptically (Figure 7).

Just as glutamate release requires multiple types of calcium channels (Luebke et al., 1993; Takahashi and Momiyama, 1993; Castillo et al., 1994; Wheeler et al., 1994), our results suggest that the same can be true for dynorphin release, since dendritic dynorphin release required both L- and N-type calcium channels. When multiple types of calcium channels are required for glutamate release, the sum of the inhibitory effects of selective channel blockers exceeds 100%. This observation suggests that there is not an excess of calcium channels present at the release site, and that there is a nonlinear relationship between calcium influx and transmitter release. Since the fusion of glutamate-containing vesicles is a function of intracellular calcium concentration to the third or fourth power, blocking a given proportion of the calcium influx would inhibit a much larger proportion of the glutamate release (Takahashi and Momiyama, 1993). Assuming that the receptor antagonist nBNI blocked 100% of the dynorphin-mediated depression, it is clear that the sum of the inhibitory effects of nifedipine and CgTx on dynorphin release was greater than 100%. Therefore, the release of dynorphins from dense-core vesicles may also be an exponential function of calcium concentration.

κ Receptor Action

In other areas of the nervous system, activation of κ receptors inhibits the opening of N-type calcium channels (Gross and Macdonald, 1987; Xiang et al., 1990). In the present study, however, CgTx treatment did not occlude κ receptor activation by the agonist U69593. Therefore, κ receptors in the guinea pig hippocampus are not coupled exclusively to N-type calcium channels. These κ receptors may be coupled to other presynaptic calcium channels, or they may have effects that are independent of calcium channels. A growing body of evidence indicates that κ receptors can couple to potassium channels (Fletcher and Chiappinelli, 1993; Grudt and Williams, 1993), including a G protein–activated inward rectifier (Henry et al., 1995). The opening of this inward potassium conductance would stabilize the membrane potential and thereby reduce excitability.

In summary, we have found that L-type calcium channels in the dentate gyrus mediate dendritic release of dynorphin neuropeptides, and in this manner they may play a role in important hippocampal functions such as learning and memory. Furthermore, our results demonstrate that granule cells release dynorphins from separate subcellular domains via distinct calcium-dependent mechanisms.

Experimental Procedures

Dentate Gyrus Recordings

Guinea pig hippocampal slices (600 μm) were prepared as described (Drake et al., 1994) and perfused with Krebs bicarbonate buffer (125 mM NaCl, 3 mM KCl, 4 mM $CaCl_2$, 4 mM $MgCl_2$, 1.25 mM Na_2HPO_4, 26 mM $NaHCO_3$, 10 mM glucose, saturated with 95% O_2/5% CO_2). The buffer also contained 10 μM bicuculline and, in some cases, 50 μM APV. The recording pipette was filled with 3 M NaCl and placed in the outer half of the molecular layer. Stimulating electrodes were placed in the molecular layer and layer 2 of the hilus. Perforant path was stimulated every 10–60 s at an intensity that evoked a response about half the amplitude of that evoked by 150 μA. After a stable baseline was established for 10 min, hilar HFS (HFS 1) was delivered: 300 μA, 0.3 ms square wave, 50 Hz for 1 s every other second, six times. In those slices demonstrating at least a 20% depression lasting 2–15 min, a second HFS (HFS 2) was delivered 35–45 min after HFS 1.

For the LTP experiments, a stimulating electrode was placed in the outer dentate molecular layer, and a recording pipette was placed in the stratum granulosum. Perforant path was stimulated every 60 s at a stimulation intensity that evoked a half-maximal response, and population spike amplitude (peak to peak) was measured. At least 15 min after drug application and after 10 min of stable baseline recording, LTP was induced. Inducing stimuli consisted of three 100 Hz, 20 or 500 ms trains of 300 μA, 0.3 ms pulses, given 1 train every 10 s.

Following LTP induction, population spike responses were again recorded at 60 s intervals for 30 min. The mean of the last five measurements was used to determine the percentage baseline value.

All drugs were bath-applied. Since the effects of CgTx are irreversible (Castillo et al., 1994; Plummer et al., 1989), a brief (20 min) application was used to block N-type channels. Statistical analyses were conducted using two-way ANOVA with one or two repeated measures, as appropriate, and the least significant difference test for post-hoc comparisons.

CA3 Recordings

Slices were perfused with Krebs bicarbonate buffer (120 mM NaCl, 3.5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgCl_2$, 1.25 mM Na_2HPO_4, 26 mM $NaHCO_3$, 10 mM glucose, saturated with 95% O_2/5% CO_2 and containing 50 μM APV). The recording pipette was placed in stratum lucidum of CA3. The stimulating electrode was placed in the dentate stratum granulosum at a location that produced robust paired-pulse potentiation. Single stimuli were delivered as described above. After a stable baseline was established for 10 min, LTP was induced: 100 Hz for 1 s, every 21 s, four times at baseline intensity (Castillo et al., 1994). The first dynorphin-releasing HFS was delivered 30 min after LTP induction, and the second was delivered 35–45 min after the first. After the effect of CgTx had stabilized, stimulus intensity was increased to produce a fEPSP amplitude similar to that before CgTx treatment (see Figure 4D, c and d). The glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM) was applied at the end of a subset of experiments to confirm that CgTx did not eliminate the synaptic component of the waveform (Figure 4D, f).

Materials

Bicuculline methiodide (Sigma), APV, CNQX, and nBNI (Research Biochemicals International) were diluted in water at stock concentrations at least 1000-fold greater than the desired final concentration. U69593 (Research Biochemicals International) was diluted first in 50% ethanol; subsequent dilutions were in aqueous solution, and the concentration of ethanol applied to the slice was ≤0.05%. Nifedipine (Sigma) and isradipine (Research Biochemicals International) were diluted in dimethylsulfoxide at stock concentrations at least 1000-fold greater than the desired final concentration. Nifedipine was protected from light at all times. CgTx (Peptides International) was dissolved in water and applied with 1 mg/ml cytochrome C (Sigma) in buffer.

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